Metabolism of Heme and Bilirubin in Rat and Human Small Intestinal Mucosa

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ABSTRACT Formation of heme, bilirubin, and bilirubin conjugates has been examined in mucosal cells isolated from the rat upper small intestine. Intact, viable cells were prepared by enzymatic dissociation using a combined vascular and luminal perfusion and incubated with an isotopically labeled precursor, δ-amino-[2,3-³H]levulinic acid. Labeled heme and bile pigment were formed with kinetics similar to those exhibited by hepatocytes. Moreover, the newly formed bilirubin was converted rapidly to both mono- and diglucuronide conjugates. In addition, cell-free extracts of small intestinal mucosa from rats or humans exhibited a bilirubin-UDP-glucuronyl transferase activity that was qualitatively similar to that present in liver. The data suggest that the small intestinal mucosa normally contributes to bilirubin metabolism.

INTRODUCTION

Individual aspects of heme and heme protein metabolism in small intestinal mucosa have been the object of several recent studies. The heme proteins known as cytochrome P-450 have been measured (1, 2) and shown to be responsive to factors in the diet (3) or to administered drugs (4, 5). As in liver, they mediate mixed-function oxygenation reactions and, therefore, are important in the biotransformation of certain orally administered drugs (6, 7). Their presence presumably reflects endogenous heme synthesis, although little is known of this process in the small intestine.

Degradation of heme to bilirubin and iron in intestinal mucosa has long been of interest because of evidence that heme is an important source of iron in humans and is metabolized by a route different from that for inorganic iron (8). The necessity for a heme-splitting activity in the release of free iron has been postulated, and, recently, heme oxygenase activity in mucosal extracts was demonstrated (9). The activity in vitro is similar to that present in liver and is increased in young rats maintained on an iron-deficient diet (9). It remains to be determined whether it is directed primarily towards breakdown of luminal (dietary) heme, endogenous mucosal heme, or both.

Finally, the possibility that bilirubin may undergo conjugation in the small intestine has been raised, a process which if present could have important effects on the enterohepatic circulation of bilirubin and, hence, on the plasma bilirubin concentration. Although bilirubin UDP-glucuronyl transferase activity may be present in mucosal extracts from the gut (10, 11), the data are preliminary or indirect, and studies with intact cells are lacking.

We have recently adapted the technique of intravascular collagenase perfusion for preparing intact cells from rat small intestine (12). The method yields, in sequence, villus-tip, mid-villus, and lower-villus/crypt fractions, which have been characterized as to their viability and specific biochemical markers (12). With these isolates, we examine heme synthesis and the conversion of heme to bilirubin and bilirubin conjugates. Heme and bilirubin metabolism in rat small intestine appear to be similar in several respects to that in liver, including formation of bilirubin mono- and diglucuronide. The latter finding is extended to man by the demonstration that extracts of human small intestinal mucosa contain bilirubin UDP-glucuronyl transferase activity.

METHODS

Island, NY). Solutions were sterilized by filtration through a 0.45-μm membrane from Nalge Co., Nalgene Labware Div., (Rochester, NY). δ-Amino [2,3-³H]levulinic acid (29 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Penicillin G potassium was from Pfizer, Inc. (Belmont, CA), sterile streptomycin sulfate, USP, from Eli Lilly & Company (Indianapolis, IN).

Buffer solutions were as follows: balanced salt solution (BSS)[1] contained 8 g NaCl, 0.4 g KCl, 0.09 g Na_2HPO_4, 0.06 g KH_2PO_4, corrected to pH 7.3 with 0.5 N NaOH, and brought to 1 liter with distilled water; it was sterilized by autoclaving.

Tris-KCl-glycerol buffer contained 50 mM Tris–HCl (pH 7.8), 150 mM KCl, 20% (vol/vol) glycerol, and 25 IU heparin.

Phosphate-buffered saline (PBS) contained 136 mM NaCl, 4.7 mM KCl, 1.3 mM Na_2HPO_4, and 0.44 mM KH_2PO_4 (pH 7.4).

Isolation of small intestinal epithelial cells. Male Sprague-Dawley rats, 200–240 g, and male homozygous Gunn rats, 200–240 g, were allowed free access to food and water and housed under controlled lighting (12 h on, 12 h off). Enzymatic dispersion of mucosal cells from the small intestine was carried out as described (12). In brief, a 20-cm segment of jejunum was perfused via catheters in the thoracic aorta and portal vein with BSS containing 5.5 mM D-glucose, 6 mM glutamine, 0.4 U/100 ml insulin, 0.01% (wt/vol) bovine albumin, 1,000 U/ml penicillin, and 0.04% (wt/vol) bacterial collagenase. After 15 min, the segment was perfused intraluminally with 50 ml BSS containing 2% (wt/vol) sucrose, 1% (vol/vol) calf serum, 100 U/ml penicillin, and 50 μg/ml streptomycin (BSS-sucrose), to harvest villus-tip cells. The luminal perfusion was repeated after 25 and 35 min of collagenase perfusion to obtain isolates enriched in mid-villus and lower-villus/crypt cells, respectively. Cells were washed in BSS-sucrose (70 g, 2 min) and resuspended in complete cell culture medium (modified Medium 199) with insulin 4 mU/ml, L-ascorbate 0.3 mM, D-glucose 5.5 mM, L-glutamine 5 mM, L-arginine 0.4 mM, corticosterone 0.001 mM, penicillin 100 U/ml, streptomycin 50 μg/ml, and 20% (vol/vol) calf serum, then filtered through three layers of cotton gauze and washed again in complete medium without calf serum but with 1% (vol/vol) rat serum. Isolates were examined microscopically for purity and viability, the latter tested with trypan blue (12).

Cells were finally suspended in the last medium by gentle pipetting and incubated on collagen-coated plastic dishes (2×10^6 cells/ml) in humidified 2% CO_2/air atmosphere at 37°C. When noted, studies were conducted with the individual villus-tip, mid-villus, and lower-villus/crypt isolates; otherwise, the fractions were pooled and studied as a combined mucosal isolate.

Results are expressed per milligram total cell protein. In preliminary experiments, the ratio of DNA to protein (micrograms DNA per milligrams protein) in cell isolates was determined and was 80, 90, and 86 for villus tip, mid-villus, and crypt cells, respectively, indicating that the amount of protein per cell was similar in all fractions.

Hepatocyte suspensions. Liver perfusion and hepatocyte preparation were carried out as described (13). Suspensions were placed in culture plates, incubated under the same conditions as described for small intestinal epithelial cells, and used for experiments immediately.

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[1] *Abbreviations used in this paper:* ALA, δ-aminolevulinic acid, BSS, balanced salt solution; PBS, phosphate-buffered saline.

Formation of labeled heme and bile pigments in culture. Studies were conducted with either radiolabeled δ-aminolevulinic acid (ALA) or glycine as heme precursor, and all manipulations involving bile pigments were carried out in subdued light. The label was introduced with the medium as the cells were transferred to culture plates. At designated time points, plates were put on dry ice. Frozen cells and medium were removed from the culture plate with a Teflon scraper and transferred to a conical extraction tube (50 ml, glass stoppered). After addition of 0.05 ml carrier pigment in the form of normal rat bile containing $\sim$ 15 mg/dl total bilirubin, mono- and diglucuronide conjugates were converted quantitatively to the corresponding methyl esters by alkaline methanolysis (14). Heme, unconjugated bilirubin, and the bilirubin methyl esters were extracted into chloroform (14), and the solution was reduced to dryness under nitrogen. The residue was dissolved in a small volume of chloroform and, under a constant stream of nitrogen, applied to thin-layer plates (silica gel 60, Merck Chemical Div, Merck & Co., Inc., Rahway, NJ) that were immediately developed in one of two different solvent systems.

Chloroform/methanol/acetic acid (97:2:1) was used for separation of bilirubin and its mono- and dimethyl esters (14). In this system, heme migrates < 1 cm. To separate heme from the origin, a second system consisting of chloroform/methanol/water/acetic acid (20:10:2:0.5) was used (15). Under these conditions heme migrates with Rf = 0.65, whereas bilirubin and its methyl esters migrate in a single band with Rf = 0.9. Labeled bands were removed from the plates by scraping, transferred to counting vials, and mixed with 200 μl 0.1 N NaOH and 100 μl 30% H_2O_2 to dissolve and bleach the pigments. After addition of 10 ml Dimilume-30 (Packard Instrument Co., Inc., Downers Grove, IL), radioactivity was determined by liquid scintillation spectrometry; counting efficiencies were calculated using the external standard method and did not vary significantly among the samples of individual experiments. Crystallization with carrier was used (16) when [2-¹⁴C]glycine was used as the labeled precursor because extraneous labeled cellular material interfered with the thin-layer chromatography determination. As reported (15), recovery of heme and bile pigment from culture medium of all suspensions was 95–98%, and triplicate samples varied <5% with either thin-layer chromatography system.

Bilirubin mono- and diglucuronide synthesis in subcellular fractions of small intestinal epithelial cells. From rats fasted overnight, 20-cm segments of duodenum/jejunum (measured from the pylorus), of ileum (measured from the end of the small bowel), and of colon (measured from the cecum) were resected and perfused with PBS containing 2% (vol/vol) calf serum. Human small intestine (jejunum), obtained under a protocol approved by the institutional Committee on Human Research, was from healthy young adults who required partial small bowel resection after gunshot wounds to the abdomen. Samples were immersed immediately in ice-cold PBS containing 2% (vol/vol) calf serum to wash away mucus and blood. Both rat and human specimens were processed with scraping the mucosal layer with a glass slide. The scraped mucosa was suspended in Tris-KCl-glycerol buffer (20%, wt/vol) and disrupted in a nitrogen bomb (Kontes Co., Vineland, NJ) at 850 psi for a period of 5 min and at 4°C. Enzymatically isolated rat small intestinal cells or hepatocytes were suspended in the same buffer and homogenates were prepared by disruption under nitrogen as described above.

Microsomes were prepared from the various homogenates by differential centrifugation. Bilirubin glucuronyl trans-

ferase activity in microsomes or cell homogenates was assayed as described by Blanckaert et al. (17), except that digitonin activation of cell extracts was omitted, and UDP-N-acetylglucosamine was added (18). Radiolabeled bilirubin substrate was prepared biosynthetically (19) and used in assays at concentrations in the range 0.5 to 5.0 μM. The reaction was linear for 10 min, and the radioactivity measured in conjugated bilirubin was at least fivefold increased above background levels in all samples. Control incubations, in which UDP-glucuronic acid was omitted, yielded negligible labeled conjugated bilirubin.

Determination of UDP-glucuronic acid. Extracts were prepared by mixing 1 vol of cell suspension or homogenate with 2 vol distilled water in a conical centrifuge tube, which was immediately placed in a boiling water bath. After 5 min, centrifugation was carried out to pellet suspended material. The supernatant was removed and stored at −20°C for subsequent assay. The protein content of both pellet and supernatant was measured for an estimate of total cell protein in the sample. The UDP-glucuronic acid content in extracts was determined fluorometrically (20), and the assay was linear from 10 to 1,000 pm UDPGA. The fluorescence of the reaction product (benzo[a]pyrene-3-O-glucuronide) was measured at an excitation wavelength of 378 nm and emission wavelength of 425 nm. Results are expressed as picomoles per milligram protein.

RESULTS

Formation of heme and bilirubin in isolated small intestinal epithelial cells. In cells incubated with the heme precursor ALA, labeled heme increased at a linear rate for at least 80 min; labeled bilirubin was detectable after a lag of 10–20 min, then increased linearly and, after 60–80 min of incubation, represented 20% of the total label recovered in heme and bile pigment (Fig. 1). The characteristics of the heme precursor pool were studied with the specific goal of determining the relative incorporation of exogenous or endogenous ALA into cellular heme and inferring from this whether labeled ALA, added to the culture medium, was causing an appreciable expansion of the endogenous ALA precursor pool. This possibility was examined with a dual-label study in which cells were incubated with both [2-^{14}C]glycine and [^{3}H]ALA; the contribution of each precursor to total cellular heme was quantitated (Table I). The results indicate that, at the concentrations used, the amount of ALA incorporated (picomoles per milligram protein) was ~ 0.2% of the amount of glycine incorporated, and this proportion was essentially constant between 30 and 60 min of incubation. Thus, the concentration of ALA used in these studies appears to represent a tracer level. The relative rates of incorporation of glycine and ALA are similar to those observed in rat hepatocytes in primary culture (15), suggesting that the endogenous pool of ALA may be of comparable size in liver parenchyma and small intestinal mucosa.

Studies of mucosal subfractions. The localization of heme metabolism along the villus axis was examined
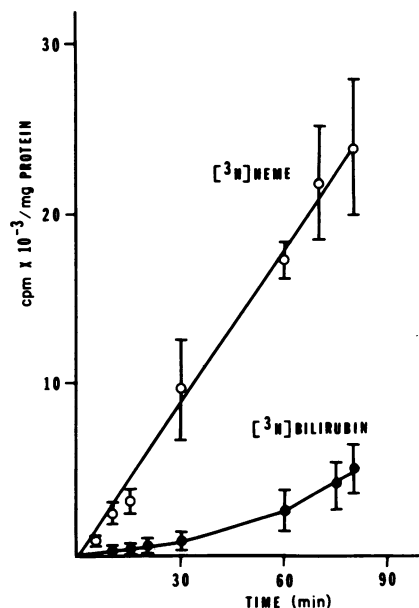

FIGURE 1 Heme and bilirubin synthesis in isolated mucosal epithelial cells from rat small intestine. Epithelial isolates (5 × 10⁶ cells) were incubated in 1 ml complete culture medium containing 1 μCi [^{3}H]ALA (29 Ci/mmol; 3.45 × 10 nM). Incubation was terminated at the indicated time points with addition of extraction solvent for measurement of radiolabeled heme and bilirubin. Results are expressed as counts per minute per milligram protein. Mean±SD ($n = 3$).

in individually isolated villus-tip, mid-villus, and lower-villus/crypt cells. Both the formation and the degradation of heme exhibited a gradient from tip to crypt, with both processes being significantly increased in the crypt cell fraction (Table II). Heme oxygenase activity similarly was least in villus-tip and greatest in crypt cells, as shown (12).

TABLE I
Contribution of Exogenous ALA to Cellular Heme Synthesis in Isolated Rat Small Intestinal Epithelial Cells

Heme precursor	Labeled heme (dpm/plate)		Precursor incorporation into heme (pmol/mg protein)	
	30 min	60 min	30 min	60 min
[^{14}C]glycine	1,610	3,296	116	237
[^{3}H]ALA	31,486	62,064	0.2	0.4

Freshly isolated epithelial cells (5 × 10⁶) were incubated in 1 ml complete culture medium containing [2-^{14}C]glycine (2.49 Ci/mol; 0.804 mM) and [^{3}H]ALA (29 Ci/mmol; 3.45 × 10 nM). After 30 and 60 min, cells and medium were removed from the plate and mixed with unlabeled carrier heme. Heme was then isolated and crystallized (16). The data represent the mean of two experiments, the individual values differing by <10%.

TABLE II
Formation of Heme and Bilirubin in Villus-tip, Mid-villus, and Lower-villus/Crypt cells

	Villus-tip	Mid-villus	Lower-villus/crypt
Heme, *dpm/mg protein*	19,537±2,344	27,911±2,737	39,570±2,915
Bilirubin, *dpm/mg protein*	3,489±392	6,226±1,622	9,274±1,604

Freshly isolated mucosal subfractions (5×10^6 cells) were incubated in 1 ml complete culture medium containing [^{3}H]ALA (29 Ci/mmol; 3.45×10 nM). After 60 min the incubation was terminated, and labeled heme and bilirubin were quantitated by thin-layer chromatography as described in Methods. Mean values±SD (n = 3).

Conjugation of bilirubin by intestinal mucosal cells. Bilirubin conjugates in isolates incubated with labeled ALA were assayed by a method that permits quantitation of the physiologic monoglucuronide conjugates of bilirubin IX-α (the C_8- or C_{12}-esters), the monoglucuronide conjugates formed nonenzymatically by dipyrrole exchange (the III-α and XIII-α forms), and also bilirubin IX-α diglucuronide (14). Of the total labeled bile pigment recovered after 60 min of incubation, monoglucuronide conjugates represented 29% and diglucuronide 24%; the remainder was unconjugated bilirubin (Fig. 2). Radioactivity present in the III-α and XIII-α monoglucuronide isomers was

<4% of the total bile pigment at any time point, suggesting that dipyrrole exchange was occurring very slowly, if at all, during the analytical procedures. The ratio of disintegrations per minute in the major monoglucuronide isomers (C_8/C_{12}) ranged from 1.4 to 1.8. Although total bilirubin formation varied among isolates from the tip, intermediate, and lower-villus areas (as shown above), the proportion of monoconjugated, diconjugated, and unconjugated bilirubin formed was similar in all (data not shown).

The specificity of the observed conjugating activity was tested in a parallel series of experiments with cells isolated from homozygous Gunn rats, in which hepatic UDP-glucuronyl transferase activity is lacking, and bilirubin glucuronide is undetectable in plasma and bile (21). In intestinal mucosal cells from these animals, formation of bilirubin mono- and diglucuronides was <1% of that present in normal cells (Fig. 3).

Bilirubin UDP-glucuronyl transferase activity and UDP-glucuronic acid content of rat and human small intestinal mucosa. Endogenously formed UDP-gluc-
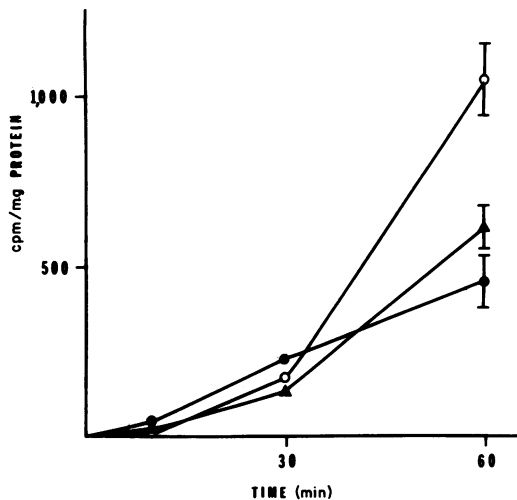

FIGURE 2 Bilirubin glucuronide formation in intact epithelial cells from rat small intestinal mucosa. Isolates (3.5×10^6 cells) were incubated in 1 ml complete culture medium containing 1 μCi [^{3}H]ALA (29 Ci/mmol; 34.5 nM). Incubation was terminated at designated time points by placing the culture plates on dry ice. After addition of carrier pigment in the form of rat bile, bilirubin mono- and diglucuronides were converted to their corresponding monomethylesters by alkaline methanolysis (14), extracted, separated, and quantitated as described in Methods. Mean values±SD (n = 3). O, [^{3}H]UCB; ▲, [^{3}H]BMG; ●, [^{3}H]BDG.
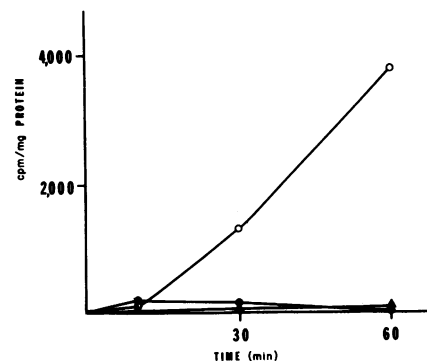

FIGURE 3 Bilirubin formation in intact epithelial cells from small intestinal mucosa of homozygous Gunn rats. Cells were incubated and processed as described in Fig. 2. Results from two individual experiments were essentially identical; the data represent the mean values. O, [^{3}H]UCB; ▲, [^{3}H]BMG; ●, [^{3}H]BDG.

uronic acid is required for conjugation of bilirubin in intact tissue. This was assayed in extracts from rat and human small intestine and found to be 0.23 and 1.99 nmol/mg protein, respectively. These concentrations are comparable to those reported for rat (22) and human liver (unpublished observations).

Microsomal extracts from three different intestinal segments—proximal small bowel, ileum, and colon—each exhibited bilirubin UDP-glucuronyl transferase activity (Table III). Although the specific activity varied among animals, the mean activities from all bowel segments were similar, in the range 40 to 65 pmol conjugate formed per minute per milligram protein. Assay of hepatic microsomes by this method yields an average value of 520 pmol/min per mg protein (17). On this basis, the capacity for bilirubin conjugation in rat intestinal mucosa appears to be ~10% of that in liver. A similar relationship was noted when homogenates of intact mucosal cells or hepatocytes were assayed, suggesting that the microsomal extract accurately reflects the activity present in cells.

While differing quantitatively, the intestinal and hepatic enzyme activities exhibited qualitative features in common. When extracts of proximal small bowel were incubated with varying concentrations of bilirubin (0.5–4.5 μM), the proportion of conjugates present as bilirubin diglucuronide fell from 19 to 13%; a similarly inverse relationship between formation of the diglucuronide species and substrate concentration was reported (17) for hepatic bilirubin conjugation albeit over a higher range of bilirubin concentrations. Also, in studies with whole cell homogenates, the ratio of radioactivity in the monoglucuronide isomers (C_8/C_{12}) was 1.3 to 1.8, from both intestine and liver.

Bilirubin conjugation in human small intestinal microsomes was assayed and was linear for at least 10 min (Fig. 4); in preparations from two patients monoglucuronide appeared at 139 and 111, and
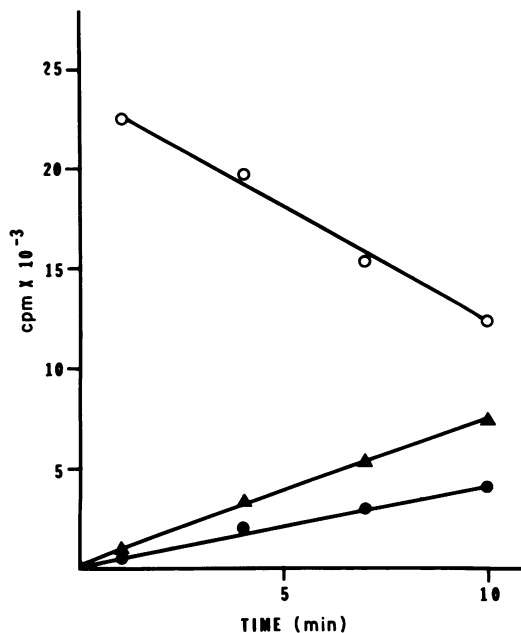

FIGURE 4 Bilirubin UDP-glucuronyltransferase activity in microsomes of human small intestine. Incubations were carried out with [^{14}C]bilirubin (5.22 mCi/mol; 4.6 μM) as substrate and 0.67 mg microsomal protein per assay. At the indicated time points, incubations were terminated by addition of methanol and carrier pigment in the form of rat bile. Bilirubin glucuronides were converted to their corresponding mono- and dimethylesters, extracted, and quantitated as described in Methods. Results are expressed as radioactivity (counts per minute) recovered after thin-layer chromatography. The data represent a typical result. O, [^{14}C]UCB; ▲, [^{14}C]BMG; ●, [^{14}C]BDG.

diglucuronide appeared at 68 and 55 pmol/min per milligram protein, respectively. Hepatic microsomes from a single patient, incubated with a low concentration of bilirubin (2.3 μM), yielded a similar value (118 pmol total conjugate formed per minute per milligram protein).

DISCUSSION

These experiments with isolated intact mucosal cells provide direct evidence for synthesis of heme, formation of bilirubin, and conversion of bilirubin to glucuronide conjugates in the small intestine. Labeled heme precursor (ALA) is converted rapidly to heme and bilirubin, with a time-course similar to that for "early labeled" bilirubin in liver (15), and its contribution (relative to that of endogenous ALA) to cellular heme synthesis is small, as in rat hepatocytes cultured under similar conditions (15). These findings are consistent with active heme metabolism in intestinal epithelium. In view of the resemblance to liver, it would be of interest to examine the regulation of ALA

TABLE III
Bilirubin Conjugation by Microsomes from Proximal Small Intestine, Ileum, and Colon

	Conjugate formed		
	BMG	BDG	Total
	pmol/min/mg protein		
Duodenum/jejunum	50±61	11±14	61±75
Ileum	31±10	9±5	40±13
Colon	59±47	6±9	65±55

Incubations were carried out as described in Methods, with 0.70 mg protein and 4.2 μM [^{14}C]bilirubin (14 mCi/mol). The data represent mean±SD of preparations from three animals. BMG, bilirubin monoglucuronide; BDG, bilirubin diglucuronide.

synthetase, the rate-determining enzyme of heme synthesis in liver, and the response of intestinal heme formation and degradation to administered drugs.

Formation of bilirubin in the small intestine, as in cultured hepatocytes, was detectable within 15 min after introduction of labeled ALA (Fig. 1), and in studies using pulse-labeling with ALA (15), degradation of newly formed heme appeared to yield stoichiometric amounts of bile pigment (unpublished observations). Bilirubin is rapidly metabolized further to mono- and diglucuronide conjugates, and, as in liver, formation of monoglucuronide appears to increase in proportion to the concentration of available substrate (unconjugated bilirubin) (23). Within the monoglucuronide fraction, the C_8 and C_{12} isomers are present in a ratio comparable to that generated by normal rat liver microsomes (18). The parallel between liver and small intestine is extended by the finding that intestinal, as well as hepatic, cells from the mutant Gunn rat form bilirubin but fail to conjugate it. In human tissue, although studies were conducted with extracts rather than intact cells, the finding of UDP-glucuronic acid and bilirubin UDP-glucuronyl transferase activity indicates that conjugation of bilirubin occurs in the small intestine of humans as well as rats.

The similarity of heme and bilirubin metabolism in liver and small intestine suggests that the latter tissue may contribute importantly to the conjugation and elimination of bilirubin from the body. The apparent specific activity of UDP-glucuronyl transferase (per milligram microsomal protein) in rat small intestine is 10% of that in liver and the mass of small intestinal mucosa is ~25% the hepatic mass (unpublished observations). This difference between hepatic and intestinal enzyme content may be substantially less in the case of the human tissues, although additional studies are needed. In any event, metabolism of bilirubin in specific compartments may be affected by the presence of glucuronidating activity within the intestinal mucosa. A portion of the unconjugated bilirubin in the intestinal lumen, primarily in the colon, undergoes absorption and reexcretion by the liver ("enterohepatic cycling") (24). It has been assumed that the species entering the circulation is unconjugated bilirubin, although direct evidence for this view is lacking (24). The present data, demonstrating bilirubin UDP-glucuronyl transferase activity in distal as well as proximal bowel, provide strong circumstantial evidence that bilirubin is conjugated as it traverses the intestinal mucosa, a process which in all likelihood would affect its space of distribution and half-life within the body (25). Similarly, bilirubin passing from plasma to the intestinal lumen (26) may undergo conjugation to be retained within the bowel and excreted more efficiently than unconjugated bilirubin. Further studies are needed to substantiate these possibilities.

Examination of heme and bilirubin formation in villus subfractions suggests that heme turnover is most prominent in the lower-villus/crypt region both by measurement of heme oxygenase activity in cell extracts and bilirubin formation in intact cells. By contrast, the major heme protein of the intestine, cytochrome P-450, is present in greatest concentration in villus-tip cells and least in lower-villus/crypt cells (12). Thus, a reciprocal relationship appears to exist between the heme protein content and heme oxygenase activity of mucosal subfractions. With experimental manipulations, also, such as dietary iron deficiency, reciprocal changes in cytochrome P-450 and heme oxygenase have been observed (9, 27). These findings closely resemble those reported earlier for liver (28), suggesting that endogenous heme, derived from cytochrome P-450, induces heme oxygenase, albeit results at variance with this interpretation have been reported (29).

Enzymatic cleavage of the heme ring presumably is required before heme iron is released and available for transfer to plasma and use by the organism. The distribution of heme oxygenase activity in mucosal fractions suggests that the lower-villus/crypt region may be particularly important in mediating the assimilation of heme iron. The present findings indicate also that not only the degradation but the turnover of endogenous heme is increased in lower-villus/crypt cells. If heme degradation results in transfer of iron out of the cell, then ongoing synthesis of heme implies input of iron, presumably from the intestinal lumen. It is possible that this process constitutes a pathway for the transfer of iron from lumen to plasma involving its transient organification as heme.

Synthesis of heme, its degradation to bile pigment, and conjugation of bilirubin are metabolically linked processes that may well be involved with formation of heme enzymes and transport of iron, as outlined above. Related questions concern the uptake of dietary heme and inorganic iron from the lumen and modulation of these processes at the cellular level in iron deficiency or pathologic iron excess (hemochromatosis). Further study of these areas is needed, and the availability of viable, isolated mucosal subfractions provides a direct approach.

ACKNOWLEDGMENTS

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